FINAL REPORT

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Personal Endotoxin Exposure in School Children with Asthma

Submitted by

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ABSTRACT

Exposure to endotoxin has been associated with exacerbation of asthma and with increased asthma prevalence. However, there is little data regarding personal exposure to endotoxin and limited data on the relation of personal endotoxin exposure to indoor and outdoor home, or ambient airborne endotoxin exposures. We characterized personal exposures to endotoxin in 45 school children with asthma ages 9-18 years using 376 repeated measurements from a $PM_{2.5}$ active personal exposure monitor. We also assayed endotoxin in 680 $PM_{2.5}$ samples collected from central sites and from 12 indoor and outdoor home sites in Riverside and Whittier, CA. The only significant predictor of personal endotoxin in mixed regression models was ambient endotoxin in the model including both regions. Neither indoor nor outdoor home endotoxin was a significant predictor of personal endotoxin. We found small positive correlations of personal endotoxin with personal $PM_{2.5}$ EC and OC, but not total $PM_{2.5}$ mass. Dog ownership was significantly associated with personal but not indoor endotoxin. We conclude that it may be insufficient to assume that any fixed site measurement of endotoxin adequately represents personal exposure, including measurements in the home environment. This conclusion applies to short-term exposures that may be involved in the acute exacerbation of asthma.

EXECUTIVE SUMMARY

Background:

Endotoxin is a cell wall component of in the outer membrane of gram negative bacteria. Sources include animals and agricultural activities. Exposure to endotoxin has been associated with increased airway inflammation, with exacerbation of respiratory allergic diseases including asthma, and with increased asthma prevalence. However, there is little data regarding personal exposure to endotoxin since most studies have relied on crude estimates of exposure using vacuumed house dust samples. There is also limited data on the relation of personal endotoxin exposure to indoor home, outdoor home, or ambient airborne endotoxin. There is no data to our knowledge on the relation between personal endotoxin and personal or ambient air pollution exposures. The impact of dog ownership (a major source of residential endotoxin) on personal exposure to endotoxin is also unknown.

Methods:

We characterized personal exposures to endotoxin in school children ages 9-18 years with asthma using 376 repeated measurements from $PM_{2.5}$ quartz filters collected in another study funded by NIH, NIEHS (grant no. ES011615). The present study included endotoxin assays and analysis of personal particle samples from 45 asthmatics children with complete personal air pollutant exposures and health outcome data to provide supplemental endotoxin data for future research. Subjects carried an active personal exposure monitor for $PM_{2.5}$ mass, $PM_{2.5}$ EC, $PM_{2.5}$ OC, NO_2, temperature and relative humidity during one of the 12 exposure assessment periods of 10 days duration. In addition, for the present study we assayed endotoxin in 680 $PM_{2.5}$ quartz filters plus field blanks collected from central ambient sites in Riverside and Whittier, CA, and from 12 indoor and outdoor home sites. Endotoxin was measured using the Limulus Amoebocyte Lysate kinetic chromogenic assay. $PM_{2.5}$ mass, $PM_{2.5}$ EC, and $PM_{2.5}$ OC were also measured at all of those stationary monitoring sites under previous funding. Ambient data for O_3 and NO_2 was available from the South Coast Air Quality Management District.

We first assessed the relationship between personal endotoxin exposures, and simultaneous personal exposure to $PM_{2.5}$ mass, $PM_{2.5}$ EC, $PM_{2.5}$ OC, and NO_2. We then assessed the relationship of personal endotoxin exposures to central site measurements of ambient $PM_{2.5}$ endotoxin and the same air pollutants plus O_3 (for all subjects), and to indoor and outdoor home $PM_{2.5}$ endotoxin, $PM_{2.5}$ mass, $PM_{2.5}$ EC, and $PM_{2.5}$ OC (for a subset of 14 subjects in 12 homes). We also assessed the relation between dog ownership and personal and indoor endotoxin levels. We used both Spearman rank correlations and mixed linear regression models to assess relations between exposures. Because of substantial differences in findings, most analyses are presented separately for Riverside and Whittier.

Results:

Although arithmetic means of personal endotoxin were higher for both regions, geometric means were lower and substantially so compared with all stationary sites. We found personal endotoxin in both Riverside and Whittier was not significantly correlated with indoor endotoxin or with any of the indoor air pollutants. Personal endotoxin was also not significantly correlated with outdoor endotoxin in either Riverside or Whittier. We observed small positive correlations between personal and ambient endotoxin in Riverside but not Whittier.

In correlation analyses of endotoxin and air pollution, we found personal endotoxin showed small inverse correlations with personal $PM_{2.5}$ mass and small positive correlations with personal $PM_{2.5}$ EC and OC, especially in Whittier, which has a greater impact of local traffic. We found mostly negative or null correlations of personal endotoxin with outdoor home and ambient air pollutants. Indoor endotoxin in was positively correlated with indoor $PM_{2.5}$, EC and OC in both regions. Outdoor home endotoxin as well as ambient endotoxin was positively correlated with these pollutants and with NO_2 in Whittier only.

We also found that personal endotoxin exposure levels and correlations with temperature varied between the two regions, which are characterized by large differences in weather. This may be responsible for the finding of indoor to outdoor endotoxin ratios that were opposite between the two sites, with a ratio < 1.0 at Riverside (0.48), and a ratio > 1 at Whittier (2.19). There were positive linear relations between log transformed indoor and outdoor home endotoxin in both regions (R^2 0.25 Whittier, R^2 0.28 Riverside).

In regression models, the only significant predictor of personal endotoxin was ambient endotoxin in the model including both regions. Neither indoor nor outdoor home endotoxin was a significant predictor of personal endotoxin. In the regression analysis of the relation between personal endotoxin and dog ownership, for each dog owned, personal endotoxin exposure approximately doubles. The number of dogs was not associated with indoor endotoxin.

Conclusions:

Our results suggest that it may be insufficient to assume that any fixed site measurement of endotoxin adequately represents personal exposure, including measurements in the home environment. Given that our analysis was based on daily exposures using measurements all conducted with active 24-hour samplers, this conclusion firmly applies to short-term exposures that may be involved in the acute exacerbation of asthma. The association between personal (but not indoor) endotoxin and dog ownership supports the view that personal dust cloud exposures may be the predominant driver of personal endotoxin exposure. The correlation between endotoxin and traffic-related pollution also suggest that common sources in dust and/or shared meteorological determinants are important sources of PM exposure. We also provide evidence that regional differences, including weather, are important to consider in assessing endotoxin exposures. The information provided in this study will support design development for additional research involving both multi-pollutant and bioaerosol monitoring in cohorts of subjects with asthma to assess the potential health impacts of combined exposures.

BODY OF REPORT

1. INTRODUCTION

1.1. Scope and Purpose of the Project

Personal endotoxin exposure likely includes a substantial contribution from both indoor and outdoor sources as well as other microenvironments and personal cloud exposures. We aimed in this study to assess these potential sources of endotoxin exposure. We characterized personal exposures to endotoxin in 45 school children with asthma using 376 repeated measurements from $PM_{2.5}$ quartz filters plus filed blanks. In the proposed work to assess personal endotoxin exposure, we used all available personal particle samples from the 45 asthmatics children with complete personal exposure monitor (PEM) and health outcome data, including exhaled nitric oxide (a biomarker of airway inflammation). In addition, we assayed 680 $PM_{2.5}$ quartz filters plus field blanks collected from central ambient sites in Riverside and Whittier, CA, and 12 indoor and outdoor home sites. In statistical analyses, we assessed the relationship between personal endotoxin exposures and concurrent endotoxin levels at ambient sites (for all 45 subjects) indoor and outdoor home sites (for a subset of 14 subjects in 12 homes). We also assessed the relationship between personal endotoxin exposures and concurrent personal exposure to $PM_{2.5}$ mass, $PM_{2.5}$ EC, $PM_{2.5}$ OC, O_3 and NO_2. We then assessed the relationship of personal endotoxin exposures to central site (ambient) measurements of the same air pollutants, and to indoor and outdoor home $PM_{2.5}$ mass, $PM_{2.5}$ EC, and $PM_{2.5}$ OC.

The following tasks were completed:

1) Aqueous extraction of quartz filters;
2) Endotoxin analysis of the filter extracts; and
3) Statistical analyses of the relation of personal to microenvironmental and ambient endotoxin and air pollution.

1.2. Background

Endotoxin is a cell wall component of in the outer membrane of gram negative bacteria. Sources include animals and agricultural activities. In its purified form, it is known as lipopolysaccharide, which is both toxic and immunogenic (Rylander 2002). A small and variable mass fraction of fine (respirable) particles ($PM_{2.5}$) includes endotoxin. Exposure to endotoxin has been associated with exacerbation of respiratory allergic diseases including asthma (Liu 2004; Rylander 2002), and with increased asthma prevalence (Thorne et al. 2005). One published epidemiologic study by Ryan et al. (2009) showed a significant positive interaction between house dust endotoxin and estimated exposure to traffic-related air pollution during the first year of life in relation to risk of persistent wheeze at age 3 years. Experimental inhalation of endotoxin in humans leads to airway inflammation, characterized by activation and migration of neutrophils (Thorn 2001). Despite its potential importance in particle-related respiratory health effects, only one study has assessed the impact of personal airborne endotoxin exposure on acute asthma status (Rabinovitch 2005). It is also the only study to have evaluated whether personal endotoxin exposure relates to microenvironmental endotoxin levels.

Rabinovitch et al (2005) followed a panel of 24 school children with asthma with personal exposure monitors (PEM) operated at 2 L/min over 24 hours for 164 person-days. They found that personal $PM_{2.5}$ or PM_{10} endotoxin exposure was associated with a decreased in expiratory lung function and increased symptoms. Geometric mean personal endotoxin was higher than indoor or outdoor school levels and was not correlated with these stationary site measurements. This finding points to the importance of personal dust cloud exposures. No other personal air pollutant data was presented in the paper. The present study will test the consistency of the exposure assessment findings of Rabinovitch et al (2005) in a larger cohort panel of 45 children with asthma followed over up to 10 days (376 person-days of endotoxin data collected with PEMs run at 4 L/min). We also have

complete ambient endotoxin measurements, and indoor and outdoor home endotoxin measurements in a subset of 14 children.

2. OVERVIEW OF THE PROJECT

The present exposure assessment study is an enhancement of the published findings related to our work under NIH, NIEHS funding (grant no. ES011615) (Delfino et al 2006, 2008). We conducted a longitudinal study with 10 daily repeated measurements of health outcomes and exposures in a panel cohort of school children with diagnosed persistent asthma who were ages 9-18 years, nonsmoking, and unexposed to environmental tobacco smoke in the home. The first panel was conducted in Riverside, CA, from August through mid-December 2003. This is a down-wind smog receptor site, which is a result of being just inland from LA County. The second panel was conducted in Whittier, CA from July through November 2004. This is a region of eastern LA County that is immediately down-wind of vehicular emission sources.

The Institutional Review Board of the University of California, Irvine approved the study protocol. Informed written consent was obtained from all subjects and one of their legal guardians. Subjects who were 9 to 18 years of age were recruited through notification of parents by local public schools. We recruited only subjects with mild to moderate persistent asthma.

The present study focused on assessing endotoxin exposures in 45 subjects who had reliable data for asthma outcomes including exhaled nitric oxide, which is a biomarker of airway inflammation (Delfino et al 2006). This data included the first four 10-d runs in Riverside involving 13 subjects and all eight 10-day runs in Whittier for 32 subjects and combined with data from Riverside. Table 1 shows the characteristics of the 45 subjects. The expected predominance of asthma among males vs. females is seen in this population. This was a diverse population with a majority of subjects identifying themselves as Hispanic along with 5 black subjects.

Table 1. Study group characteristics, Riverside and Whittier, CA asthma panels.

Subject Variables	Data
Age, mean (range)	13.5 (9-18 yr)
Gender No. (%)	
Female	14 (31)
Male	31 (69)
Race No. (%)	
Hispanic	26 (58)
White	14 (31)
Black	5 (11)
No. (%) with mean percent FEV_1 < 80%[a]	11 (24)
Asthma exacerbation last 12 mo. required hospital admission, ED visit, or clinic visit	
0 times	12 (27)
1-4 times	19 (42)
> 4 times	14 (31)

[a] Predicted from NHANES III (Hankinson et al., 1999) using two months of thrice daily home spirometry, acceptable and reproducible maneuvers.

3. MATERIALS AND METHODS

3.1 Exposure Assessment

3.1.1 Personal Exposure Monitor (PEM):

Subjects carried a PEM during one of the 12 exposure assessment periods of 10 days duration. Three to four different subjects were followed in each of the 12 periods. Subjects were followed-up daily in their homes to download data and change PEMs. Each subject wore the PEM during waking hours in a backpack and kept the backpack in close proximity off the ground (e.g. nightstand) when it was not possible to wear it (Figure 1). The air inlets for the PEM were placed over the shoulder strap so that they were close to the breathing zone when worn. The backpack was sound insulated and had an extra compartment for books to be carried during school days. Each day of the 10-day follow-up period, data from an attached motion logger (Onset Computer Corp, Pocasset, MA) was checked to assure compliance. Lack of expected motion at expected times (e.g. during known school periods), resulted in no monetary compensation to the subject for that day. This occurred on <6% of the days.

Personal measurements included real-time nephelometer mass measurements of $PM_{2.5}$ (personal DataRAM model 1200, MIE Inc., Bedford, MA) and 24-hr average EC and OC fractions of $PM_{2.5}$ collected on quartz filters using an attached filter cassette (Figure 1A). A 2.5 µm sharp-cut cyclone was attached upstream of the nephelometer and $PM_{2.5}$ for EC and OC was collected downstream at a flow rate of 4 L/min. We also measured NO_2 over 24-hr periods using a miniaturized diaphragm pump run at 0.1 L/min to sample air through triethanolamine-treated molecular sieve sorbent tubes (SKC, Fullerton, CA) (Figure 1B). We measured NO_2 based on National Institute for Occupational Safety and Health (1994) Method 6014. We also collected personal temperature and relative humidity with attached loggers (Onset Computer Corp, Pocasset, MA). Elsewhere we provide data on the validation of both the personal $PM_{2.5}$ sampler (Chakrabarti et al. 2004), and the personal NO_2 active sampler (Staimer et al. 2005).

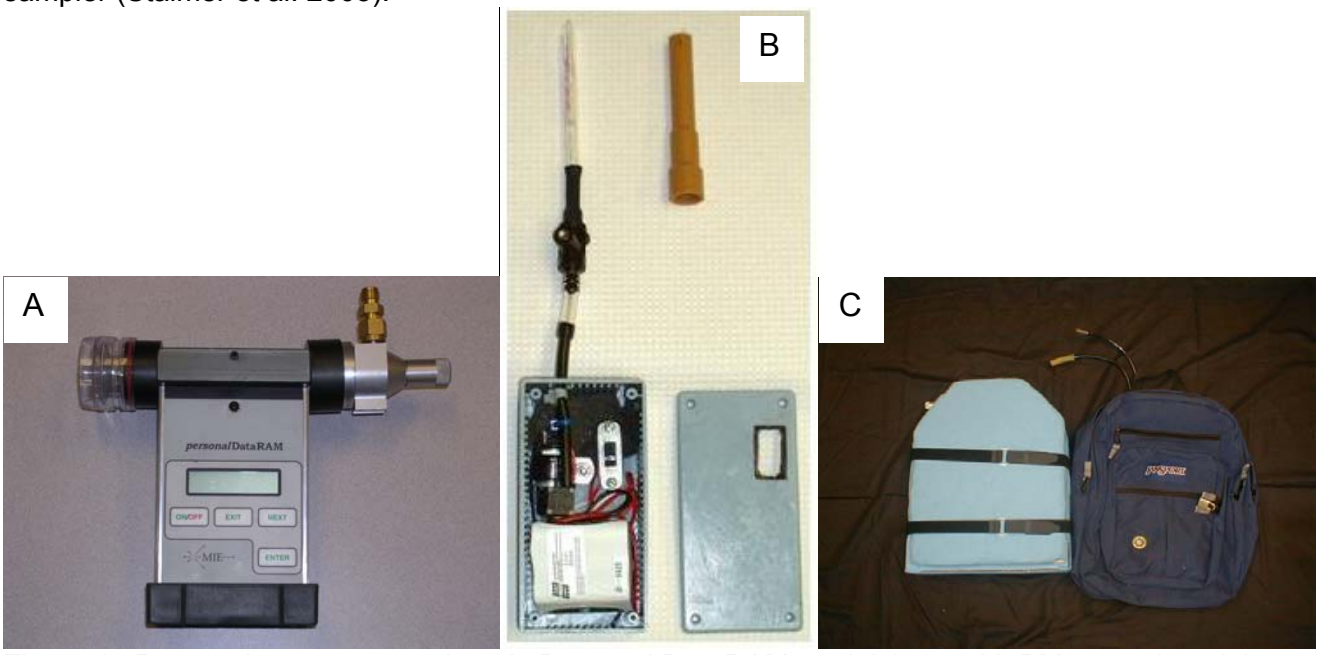


Figure 1. Personal exposure monitor. A. Personal DataRAM model 1200 with PM2.5 cyclone inlet and filter cassette at outlet (MIE Inc., Bedford, MA). B. Personal NO_2 monitor with a miniaturized diaphragm pump and triethanolamine-treated molecular sieve sorbent tubes (SKC, Fullerton, CA). C. Backpack for the PEM with sound-insulated foam encasement.

3.1.2 Stationary Site Air Monitoring:

Harvard Impactors (Air Diagnostics and Engineering, Inc., Naples, ME) were used to collect

ambient $PM_{2.5}$ and operated at a flow rate of 10 L/min. They were sited at a central site within 10 km of homes in Riverside and 5 km of homes in Whittier. We also collected indoor and outdoor home $PM_{2.5}$ with Harvard Impactors in one subject's home during each of the 12 ten-day sampling periods (Figure 2). There were a pair sibling subjects in two of the homes (yielding data for 14 subjects overall for the 12 homes). $PM_{2.5}$ (Teflon filters), and $PM_{2.5}$ EC and OC (quartz filters) were collected at the stationary sites simultaneous with personal samples. PM mass on Teflon filters was estimated using standard gravimetric methods. For both personal and stationary site quartz filter samples, particulate carbon was speciated into organic and elemental carbon using the thermal manganese dioxide oxidation technique (Fung et al. 2002). Criteria pollutant gases were measured by the South Coast Air Quality Management District at central sites and they included hourly O_3 and NO_2.

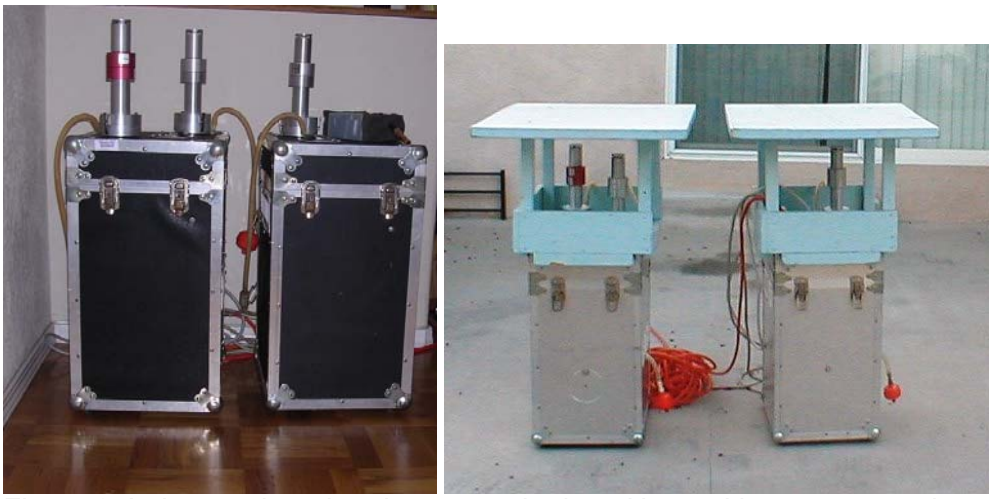


Figure 2. Indoor and outdoor home monitoring. Harvard impactor samplers (Air Diagnostics and Engineering, Inc., Naples, ME).

3.1.3 Endotoxin Assay:

Endotoxin was measured from extracts of archived $PM_{2.5}$ quartz filters (stored at -30°C) collected as described above (376 personal $PM_{2.5}$ filter samples, and 680 central site, indoor and outdoor home $PM_{2.5}$ filter samples). We do not have quartz PM_{10} samples. Although endotoxin is found in the coarse PM fraction (2.5-10 μm in diameter), the respirable $PM_{2.5}$ fraction is more relevant to lower airway dose and thus airway inflammation. All quartz filters were baked to remove organic carbon before sampling. Only around 10% of the filters' surface area was punched out using heat sterilized instruments for the EC-OC measurements, leaving sufficient filter media for endotoxin assays. The remaining surface area was calculated for each filter to estimate particle mass using mass data from the 24-hr average PEM pDR $PM_{2.5}$ for personal endotoxin or gravimetric measurements from the Harvard Impactor $PM_{2.5}$ Teflon filters for the stationary site measurements.

For the endotoxin assay, we developed a rapid and thorough method of extracting endotoxin from quartz $PM_{2.5}$ filters. Briefly, the extraction procedure combines the efficient disruption of quartz filter membranes by using a high speed, reciprocating instrument (FastPrep, MP Biomedicals, Inc., Solon, OH) with conventional sonication. Samples are then centrifuged (at 4000 rpm for 5 min, 4 °C) and an aliquot of supernatant assayed for endotoxin using the Limulus Amoebocyte Lysate kinetic chromogenic assay according to the manufacturer's protocol (Pyrochrome Associates of Cape Cod, Falmouth, MA). Negative control quartz filters (field blanks) are extracted and analyzed with each set of air samples. The detection limit for the overall method is estimated at 0.004 endotoxin units (EU)/m^3 air (non-detects are set to half this).

3.2 Analyses

Descriptive analyses of exposures were used to determine the shape of the distribution, central

tendency, and spatial trends (region). We examined the Spearman rank correlation of personal endotoxin to ambient endotoxin measured at a central site, and in the 25% subset of subjects, we assessed the rank correlation of personal endotoxin to outdoor and indoor home concentrations of endotoxin. This was intended to establish the extent to which fixed site home and regional measurements represent personal endotoxin exposure. We found notable differences in concentrations and correlations between Riverside and Whittier. Therefore, we present results separately for the two regions. Home dust samples for endotoxin were not collected because the study objective of the parent project funded by NIH, NIEHS was to assess daily acute changes in asthma outcomes.

Because the distribution of endotoxin for all measurements was log-normally distributed, we log transformed the endotoxin variables prior to all regression analyses. We first examined the relation of indoor to outdoor endotoxin in linear regression models. Multiple regression analyses of the relation of continuous log transformed personal endotoxin to stationary endotoxin measurements were conducted using the general linear mixed model. The mixed model estimates both fixed and random effects (Diggle et al. 2002) and incorporates the basic longitudinal design of the study in which multiple measurements are taken on each subject. Subject random effects (random intercepts) reflect the principle that measurements taken for the same individual are likely to be correlated (not independent).

The following *a priori* adjustments were made in the mixed models predicting personal endotoxin: personal temperature, personal relative humidity (RH), age and sex. We fit an autoregressive-1 correlation structure given the observed variability. Analyses were conducted across both regions (additionally adjusting for region) and separately by region given the difference noted above.

We also used mixed models to analyze the prediction of personal endotoxin exposure by dog ownership, including the number of dogs and whether dogs were allowed in the house (never, occasionally, or often). Dogs have been identified as a major identifiable source of endotoxin (Rylander 2002).

4. RESULTS

4.1 Descriptive analyses of endotoxin and air pollutant exposures

We found detectable endotoxin concentrations in 376 daily personal $PM_{2.5}$ filters analyzed [median 0.58, range 0.003 - 20.6 EU/m^3]. All 52 personal field blank filters showed low or non-detectable endotoxin (median 0.004 EU/filter).

We also found detectable endotoxin concentrations in all 680 Harvard Impactor filters from the stationary sites. These included 423 ambient 128 indoor and 129 outdoor home filters from active samplers succesfully extracted. The 42 blank filters at the stationary sites showed low or non-detectable endotoxin (median 0.011 EU/filter). For the comparisons with available indoor and outdoor measurements there were 117 and 114 personal endotoxin measurements, respectively, among the 14 subjects living on those 12 homes. For the comparisons with available ambient endotoxin measurements there were 344 personal endotoxin measurements among the 45 subjects. For the comparisons with available ambient air pollution there were all 376 or nearly all personal endotoxin measurements. Air pollutant measurements were nearly complete with at least 407 days for each variable available for comparison with the 423 days of ambient endotoxin measurements.

Descriptive statistics regarding all of the exposures by region are shown in Table 2. Arithmetic and geometric mean personal endotoxin exposures were higher in Riverside than in Whittier even though indoor endotoxin exposures were higher in Whittier than in Riverside. Outdoor home and ambient endotoxin was higher in Riverside than in Whittier. Although arithmetic mean personal endotoxin was higher than indoor, outdoor or ambient levels across both sites, geometric means were substantially lower than all stationary sites. This is a reflection of the typical skewed distribution of endotoxin exposures.

Table 2. Descriptive statistics of endotoxin and air pollutant exposures by region.

	Riverside				Whittier			
Exposure	**Mean (SD)**	**Geom Mean**	**IQR.**	**Min/Max**	**Mean (SD)**	**Geom Mean**	**IQR.**	**Min/Max**
Endotoxin (EU/m^3)								
Personal	2.30 (3.88)	0.58	2.25	0.003/ 20.6	1.92 (3.67)	0.28	1.97	0.002/ 25.3
Indoor	0.58 (0.42)	0.41	0.68	0.063/ 1.72	1.49 (1.29)	1.13	0.86	0.13/ 7.5
Outdoor	1.46 (1.67)	0.86	1.5	0.12/ 7.90	0.85 (1.18)	0.52	0.89	0.11/ 9.5
Ambient	1.26 (1.17)	0.91	0.88	0.30/ 4.56	0.55 (0.45)	0.40	0.46	0.048/ 2.51
Personal Exposure								
$PM_{2.5}$ (μg/m^3)	30.9 (20.1)	25.1	26.8	6.6/ 98.4	36.4 (26.8)	30.6	21.9	7.6/ 220.0
EC (μg/m^3)	0.43 (0.61)	0.32	0.32	0.04/ 6.94	0.76 (1.32)	0.39	0.82	0.001/ 17.2
OC (μg/m^3)	5.95 (2.62)	5.45	3.48	1.94/ 16.1	6.83 (3.41)	6.14	4.05	2.18/ 31.5
NO_2 (ppb)	23.3 (9.3)	21.2	12.7	5.16/ 47.6	30.6 (14.4)	27.1	18.9	2.7/ 105.7
Temperature (°F)	80.3 (3.6)	80.2	5.5	73.1/ 89.8	76.7 (4.8)	76.6	6.29	63.2/ 86.9
Relative Humidity (%)	43.9 (8.7)	43.1	14.8	28.6/ 64.0	49.8 (7.0)	49.2	7.75	25.2/ 66.6
Indoor Air Pollution								
$PM_{2.5}$ (μg/m^3)	14.9 (8.4)	12.6	14.6	5.10/ 33.8	17.4 (10.6)	15.1	8.35	3.59/ 83.2
EC (μg/m^3)	0.71 (0.30)	0.65	0.41	0.17/ 1.3	0.80 (0.92)	0.61	0.54	0.14/ 7.75
OC (μg/m^3)	6.2 (1.82)	5.98	2.74	3.17/ 11.6	5.95 (2.37)	5.55	2.27	2.64/ 13.5
Outdoor Air Polution								
$PM_{2.5}$ (μg/m^3)	27.0 (18.6)	22.2	19.4	9.3/ 71.8	19.3 (13.5)	16.4	8.46	3.18/ 84.3
EC (μg/m^3)	1.10 (0.36)	1.05	0.43	0.50/ 2.08	0.99 (1.41)	0.74	0.62	0.21/ 12.5
OC (μg/m^3)	6.2 (1.26)	6.09	1.57	3.78/ 9.66	4.54 (1.89)	4.18	2.63	2.05/ 10.3
Ambient Air Pollution								
$PM_{2.5}$ (μg/m^3)	31.5 (22.1)	25.1	30.1	9.5/ 87.2	17.8 (12.0)	15.1	8.85	2.77/ 77.1
EC (μg/m^3)	1.55 (0.71)	1.41	0.86	0.52/ 3.64	0.69 (0.44)	0.59	0.45	0.14/ 2.95
OC (μg/m^3)	6.7 (1.69)	6.49	1.91	4.11/ 11.6	3.89 (1.49)	3.62	2.07	1.64 / 8.8
NO_2 (ppb)	26.8 (9.9)	25.1	12.3	11.6/ 54.8	27.7 (10.8)	26.0	11.6	12.0/ 74.1
O_3 (ppb)	77.9 (19.7)	75.3	25.9	33.4/ 120.8	40.7 (14.1)	38.1	18.5	11.1/ 79.2
Temperature (°F)	76.0 (6.5)	75.7	10.9	63.1/ 86.2	69.3 (6.46)	69.0	9.9	53.0/ 82.1
Relative Humidity (%)	27.4 (16.2)	21.9	29	2.0/ 61.0	39.7 (9.64)	38.2	12.0	6.0/ 61.0

We show correlation matrixes separately for Riverside and Whittier relating personal endotoxin to personal air pollutants (Tables 3-4), to indoor home endotoxin and air pollution (Tables 5-6), to outdoor home endotoxin and air pollution (Tables 7-8), and to ambient endotoxin and air pollution (Tables 9-10).

In both Riverside and Whittier, personal endotoxin showed a small inverse correlations with personal $PM_{2.5}$, and small positive correlations with $PM_{2.5}$ EC and OC, although they were larger in Whittier (Tables 3-4). Personal endotoxin positively correlated with personal temperature in Riverside but negatively correlated with personal temperature in Whittier (Tables 3-4).

We also found personal endotoxin in both Riverside and Whittier was not significantly correlated with indoor endotoxin or any of the indoor air pollutants (Tables 5-6). Indoor endotoxin in Riverside, on the other hand, was strongly positively correlated with indoor $PM_{2.5}$ EC and moderately correlated with indoor $PM_{2.5}$ mass and OC (Table 5), whereas in Whittier these correlations were positive but much smaller (Table 6).

Both personal and outdoor home endotoxin in Riverside were not significantly correlated with any outdoor home air pollutant measurement (Table 7). Personal endotoxin was not significantly correlated with outdoor endotoxin in either Riverside (Table 7) or Whittier (Table 8). We observed a small inverse correlation between personal endotoxin and outdoor home $PM_{2.5}$ in Whittier (Table 8). Outdoor home endotoxin showed small positive correlation with outdoor home $PM_{2.5}$, EC and OC in Whittier (Table 8).

We observed small positive correlations between personal and ambient endotoxin in Riverside (Table 9) but not Whittier (Table 10), while at both sites there were small negative correlations between personal and ambient $PM_{2.5}$ and OC. In Whittier, ambient temperature and ozone were negatively correlation with personal endotoxin (Table 10). In Whittier, but not Riverside, ambient endotoxin showed small positive correlations with ambient traffic-related air pollutants (EC, OC, NO_2) and temperature and small inverse correlations with RH (Table 10).

Table 3. Spearman rank correlation matrix of personal exposures in Riverside, CA

	Personal Endotoxin	Personal $PM_{2.5}$	Personal EC	Personal OC	Personal NO_2	Personal Temperature	Personal RH
Personal Endotoxin	1.00	-0.24*	0.15	0.27**	0.13	0.22*	-0.04
Personal $PM_{2.5}$		1.00	-0.19	-0.18	0.56**	-0.23*	0.59**
Personal EC			1.00	0.71**	-0.002	0.31**	-0.13
Personal OC				1.00	0.03	0.60**	-0.23**
Personal NO_2					1.00	0.07	0.27**
Personal Temperature						1.00	-0.58**
Personal RH							1.00

* $p < 0.05$ ** $p < 0.01$

Table 4. Spearman rank correlation matrix of personal exposures in Whittier, CA

	Personal Endotoxin	Personal $PM_{2.5}$	Personal EC	Personal OC	Personal NO_2	Personal Temperature	Personal RH
Personal Endotoxin	1.00	-0.16**	0.40**	0.41**	-0.05	-0.41**	0.09
Personal $PM_{2.5}$		1.00	0.15*	0.21**	0.29**	0.14*	0.06
Personal EC			1.00	0.83**	0.36**	-0.47**	0.08
Personal OC				1.00	0.26**	-0.32**	0.05
Personal NO_2					1.00	-0.08	-0.12*
Personal Temperature						1.00	-0.42**
Personal RH							1.00

* $p < 0.05$ ** $p < 0.01$

Table 5. Spearman rank correlation matrix of personal endotoxin, indoor home endotoxin and air pollution in Riverside, CA

	Personal Endotoxin	Indoor Endotoxin	Indoor $PM_{2.5}$	Indoor EC	Indoor OC
Personal Endotoxin	1.00	0.10	-0.14	-0.18	-0.20
Indoor Endotoxin		1.00	0.66**	0.73**	0.40*
Indoor $PM_{2.5}$			1.00	0.78**	0.83**
Indoor EC				1.00	0.74**
Indoor OC					1.00

* $p < 0.05$ ** $p < 0.01$

Table 6. Spearman rank correlation matrix of personal endotoxin, indoor home endotoxin and air pollution in Whittier, CA

	Personal Endotoxin	Indoor Endotoxin	Indoor $PM_{2.5}$	Indoor EC	Indoor OC
Personal Endotoxin	1.00	-0.14	-0.16	-0.10	0.11
Indoor Endotoxin		1.00	0.27**	0.19	0.30**
Indoor $PM_{2.5}$			1.00	0.73**	0.71**
Indoor EC				1.00	0.71**
Indoor OC					1.00

* $p < 0.05$ ** $p < 0.01$

Table 7. Spearman rank correlation matrix of personal endotoxin, outdoor home endotoxin and air pollution in Riverside, CA

	Personal Endotoxin	Outdoor Endotoxin	Outdoor $PM_{2.5}$	Outdoor EC	Outdoor OC
Personal Endotoxin	1.00	0.21	-0.13	-0.04	-0.37
Outdoor Endotoxin		1.00	-0.03	0.35	0.09
Outdoor $PM_{2.5}$			1.00	0.48**	0.43**
Outdoor EC				1.00	0.53**
Outdoor OC					1.00

* $p < 0.05$ ** $p < 0.01$

Table 8. Spearman rank correlation matrix of personal endotoxin, outdoor home endotoxin and air pollution in Whittier, CA

	Personal Endotoxin	Outdoor Endotoxin	Outdoor $PM_{2.5}$	Outdoor EC	Outdoor OC
Personal Endotoxin	1.00	-0.19	-0.26*	-0.02	-0.07
Outdoor Endotoxin		1.00	0.23*	0.32**	0.33**
Outdoor $PM_{2.5}$			1.00	0.63**	0.72**
Outdoor EC				1.00	0.87**
Outdoor OC					1.00

* $p < 0.05$ ** $p < 0.01$

Table 9. Spearman rank correlation matrix of personal endotoxin, ambient endotoxin and air pollution in Riverside, CA

	Personal Endotoxin	Ambient Endotoxin	Ambient $PM_{2.5}$	Amb. EC	Amb. OC	Amb. NO_2	Amb. O_3	Amb. Temp.	Amb. RH
Personal Endotoxin	1.00	0.32**	-0.26*	-0.16	-0.29**	-0.25*	-0.06	0.02	0.07
Ambient Endotoxin		1.00	0.01	0.1	-0.09	-0.04	-0.14	0.13	0.18*
Ambient $PM_{2.5}$			1.00	0.24**	0.48**	0.23**	-0.18*	-0.64**	0.49**
Ambient EC				1.00	0.52**	0.83**	0.11	0.10	-0.26**
Ambient OC					1.00	0.75**	0.49**	0.16	-0.43**
Ambient NO_2						1.00	0.34**	0.33**	-0.53**
Ambient O_3							1.00	0.47**	-0.58**
Ambient Temperature								1.00	-0.83**
Ambient RH									1.00

* $p < 0.05$ ** $p < 0.01$

Table 10. Spearman rank correlation matrix of personal endotoxin, ambient endotoxin and air pollution in Whittier, CA

	Personal Endotoxin	Ambient Endotoxin	Ambient $PM_{2.5}$	Amb. EC	Amb. OC	Amb. NO_2	Amb. O_3	Amb. Temp	Amb. RH
Personal Endotoxin	1.00	-0.02	-0.24**	-0.00	-0.15*	0.04	-0.36**	-0.43**	0.02
Ambient Endotoxin		1.00	0.01	0.37**	0.36**	0.30**	0.05	0.37**	-0.39**
Ambient $PM_{2.5}$			1.00	0.65**	0.72**	0.41**	0.44**	0.25**	-0.00
Ambient EC				1.00	0.88**	0.82**	0.23**	0.26**	-0.51**
Ambient OC					1.00	0.72**	0.48**	0.44**	-0.49**
Ambient NO_2						1.00	0.012	0.06	-0.57**
Ambient O_3							1.00	0.70**	-0.14*
Ambient Temperature								1.00	-0.22**
Ambient RH									1.00

* $p < 0.05$ ** $p < 0.01$

Table 11 shows indoor to outdoor endotoxin ratios. Ratios were clearly opposite between the two sites with a ratio < 1.0 at Riverside (0.48) and a ratio > 1 at Whittier (2.19). Actual indoor concentration reflected this difference with a much smaller indoor concentration in Riverside than in Whittier.

Table 11. Indoor to outdoor endotoxin ratios.

	Geometric Means		Indoor / Outdoor Endotoxin ratio[1]
Geographic region	Indoor Endotoxin (EU/m^3)	Outdoor Endotoxin (EU/m^3)	
Both	0.88	0.59	1.49
Riverside	0.41	0.86	0.48
Whittier	1.13	0.52	2.19

[1] Ratios are calculated for displayed geometric means.

4.2 Regression analyses of endotoxin exposures

The prediction of personal endotoxin in mixed regression models by the various stationary site measurements are shown in Table 12 including both sites and by region. The only significant predictor of personal endotoxin was ambient endotoxin in the model including both regions. Neither indoor nor outdoor home endotoxin was a significant predictor of personal endotoxin.

Table 12. Associations of personal log endotoxin with indoor, outdoor and ambient log endotoxin.[1]

Predictor Variable	All Subjects coefficient (95% CI)	Riverside coefficient (95% CI)	Whittier coefficient (95% CI)
Log Indoor Endotoxin	0.09 (-0.52, 0.69)	-0.03 (-2.47, 2.42)	0.10 (-0.63, 0.83)
Log Outdoor Endotoxin	0.21 (-0.31, 0.72)	-0.44 (-2.59, 1.70)	0.26 (-0.32, 0.84)
Log Ambient Endotoxin	0.35 (0.01, 0.69)*	0.20 (-2.58, 2.97)	0.46 (-0.21, 1.12)

* $p < 0.05$

[1] Results of linear mixed effects models show the change in log personal endotoxin for a one log-unit change in the predictor variable, adjusted for age, gender, personal temperature and RH.

Figures 3-4 show scatter plots and results of a linear regression model for the relation between log transformed indoor and outdoor home endotoxin for 10-day monitoring sessions in 4 homes in Riverside and 8 homes in Whittier. In both regions, the relation was positive, with outdoor endotoxin explaining 25-28% of the variability (R^2) in indoor endotoxin.

Figure 3. Relation between indoor and outdoor home endotoxin for 10-day monitoring sessions in 4 homes in Riverside.

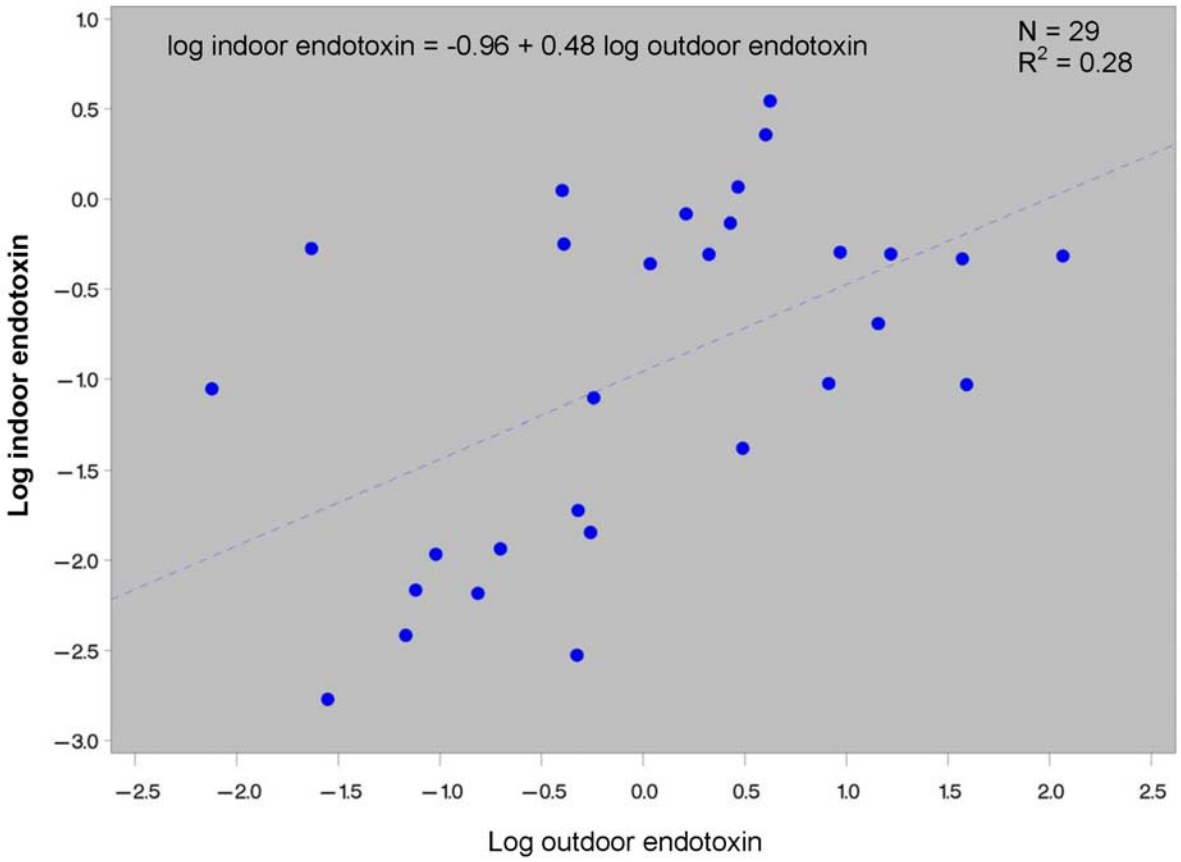
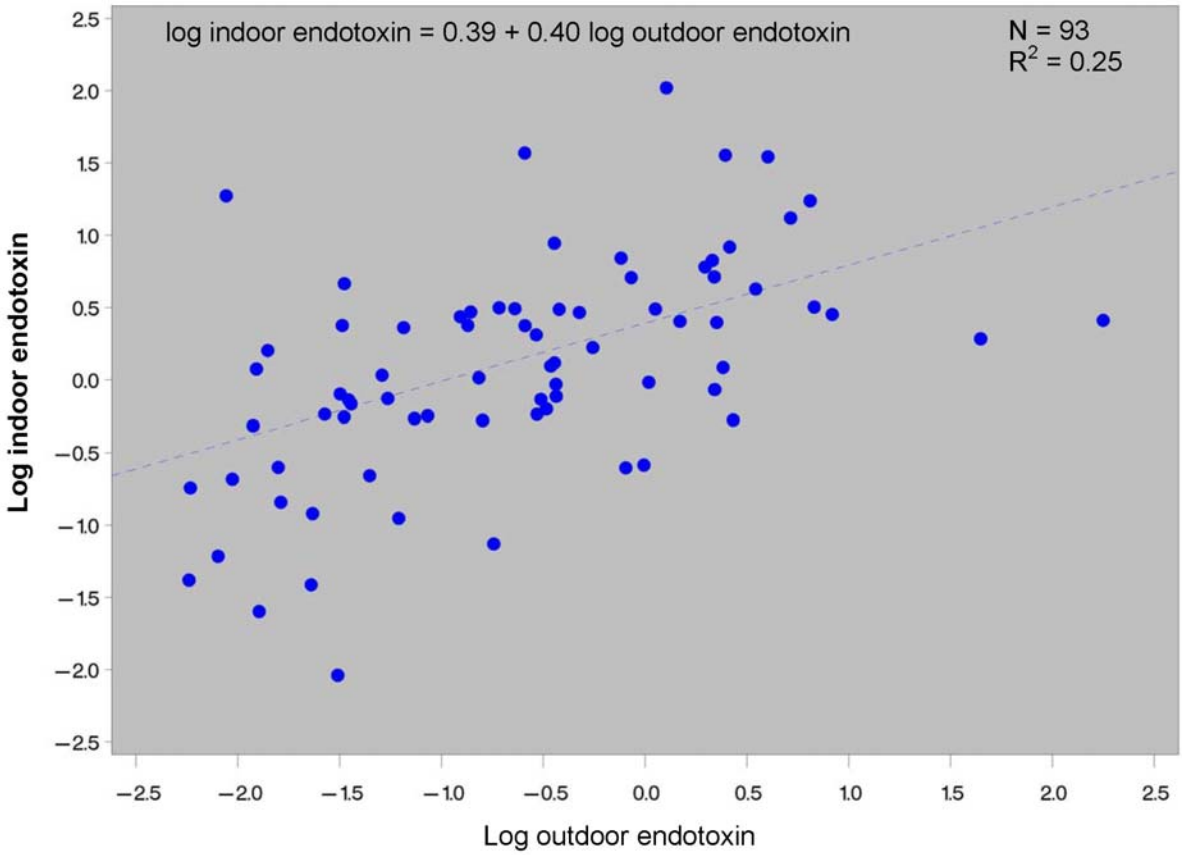


Figure 4. Relation between indoor and outdoor home endotoxin for 10-day monitoring sessions in 4 homes in Whittier.



The analysis of the relation between personal endotoxin and dog ownership reveals a clear positive association (Table 13). For each dog owned, personal endotoxin exposure approximately doubles and there is little difference between adjusted and unadjusted models. Interestingly, compared with having no dogs, the strongest association with personal endotoxin was for dogs that were only occasionally indoors followed by outdoor dogs only (nominally significant at $p < 0.1$). In contrast, compared with having no dogs, having dogs that were often indoors did not significantly relate to personal endotoxin. The number of dogs was not associated with indoor endotoxin, which was actually significantly lower by around 90% for homes with dogs often indoors compared with no dogs.

Table 13. Relation of dog ownership to personal and indoor endotoxin exposures: average proportional increase in endotoxin exposure.[1]

	Personal Endotoxin (45 subjects)		Indoor Endotoxin (12 homes)	
Predictor	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI) [1]	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI) [1]
Number of dogs	2.00 (1.36, 2.93)***	1.88 (1.38, 2.58)***	0.87 (0.55, 1.37)	0.98 (0.59, 1.63)
Residence of dogs				
No dogs	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Often Indoors	1.61 (0.46, 5.64)	1.82 (0.61, 5.41)	0.10 (0.04, 0.25)***	0.10 (0.01, 0.75)**
Occas. Indoors	5.22 (1.50, 18.2)***	3.15 (1.00, 9.92)**	0.59 (0.32, 1.06)*	0.73 (0.28, 1.90)
Outdoor Only	2.91 (0.85, 9.98)*	2.61 (0.81, 8.37)	0.80 (0.42, 1.52)	0.87 (0.30, 2.52)

* $p < 0.10$ ** $p < 0.05$ *** $p < 0.01$

[1] Because the dependent variable (personal or indoor endotoxin), we exponentiated the regression coefficient of the predictor, thus yielding the proportional or relative percentage change in endotoxin exposure

[1] Adjusted for personal temperature and relative humidity, sex age, and region (Riverside vs. Whittier)

[2] 14 subjects had no dogs, 11 had one dog, 16 had two dogs, five had 3 dogs, and one had four dogs.

5. SUMMARY AND CONCLUSIONS

Our results suggest that it may be insufficient to assume that any fixed site measurement of endotoxin adequately represents personal exposure, including measurements in the home environment and ambient central sites. Given that our analysis was based on daily exposures using measurements all conducted with active 24-hour samplers, this conclusion firmly relates to short-term exposures that may be involved in the acute exacerbation of asthma. The standard measurement of endotoxin exposure in studies of chronic asthma is to utilize vacuumed house dust samples as the sample for endotoxin testing. We did not assess whether this type of measurement is representative of long-term personal exposure. Also, endotoxin is found in the coarse PM fraction (2.5-10 μm in diameter), which we did not measure. Nevertheless, the respirable $PM_{2.5}$ fraction is more relevant to lower airway dose and thus airway inflammation. Another limitation is that the the number of indoor and outdoor home samples was limited to 14 of the 45 subjects, and this may have limited the power to assess relations of home to personal endotoxin. This was not a limitaiton for ambient endotoxin where data from all 45 subjects could be used.

Our findings of a general lack of correlation between personal and stationary site endotoxin is consistent with the findings of Rabinovitch et al. (2005). In a smaller panel of school children with asthma, they found geometric mean personal endotoxin was higher than indoor or outdoor school endotoxin levels, and personal endotoxin was not correlated with these stationary site measurements. However, we found that although arithmetic mean personal endotoxin was higher than indoor, outdoor or ambient levels across both regions of study, geometric means were lower than geometric means at all stationary sites. This is a reflection of the highly skewed distribution of personal endotoxin.

We also conclude that personal endotoxin exposure can vary between regions characterized by large differences in southern California weather, with Riverside being a hot inland area and Whittier being a milder climate with greater coastal influences. Regional differences in correlations of personal

endotoxin with both personal and ambient temperature and regional differences in indoor/outdoor endotoxin ratios may have been due to this difference in weather. However, we cannot rule out unmeasured differences in the homes of subjects between these two regions or differences in other microenvironments of the subjects, including endotoxin at schools, where we did not measure endotoxin. We did observe a positive linear relation between outdoor and indoor endotoxin that was small but significant and similar between the two regions.

We observed small positive correlations of personal endotoxin with traffic-related air pollutants ($PM_{2.5}$ EC and OC) especially in Whittier, which has a greater impact of local traffic. In Whittier, but not Riverside, ambient endotoxin also showed small positive correlations with ambient traffic-related air pollutants (EC, OC, and NO_2). These observations might be attributable to re-suspension of fine dust laden with bioaerosols along nearby roadways, which also generate higher concentrations of the traffic-related pollutants.

The results examining the relation of personal endotoxin to the number of dogs owned was largely as expected and it substantiates the utility of the personal exposure measurements. The stronger association of personal endotoxin with having dogs that were only occasionally indoors could be attributed to entrainment of debris from the outdoor environment into the indoor environment, including fecal matter. However, there was no association between indoor endotoxin and the number of dogs owned and a negative relation of indoor endotoxin for homes with dogs often indoors vs. no dogs. This last finding is unexplained and potentially spurious; however, it is conceivable that entrainment of debris from the outdoor environment was intentionally limited in homes with dogs kept primarily indoors.

Overall evidence from our analyses of a lack of prediction by fixed site endotoxin and the association between personal endotoxin and dog ownership supports the view that personal dust cloud exposure may be the predominant driver of personal endotoxin exposure. This is a phenomenon where localized personal activities lead to increased PM exposure by re-suspension of settled PM and bring the subjects into closer breathing zone contact with PM whatever the source.

Our results also shed light on our previous findings as follows. In the NIEHS-funded study we found that personal air pollutant exposures (NO_2 and $PM_{2.5}$ mass, $PM_{2.5}$ EC, and $PM_{2.5}$ OC) were associated with both decreased lung function as measured by FEV_1 (Delfino et al. 2008) and increased airway inflammation as measured by FE_{NO} (Delfino et al. 2006). We had hypothesized that the markers of exposure to pollutants from fossil fuel combustion (EC, OC and NO_2) would be more strongly and precisely associated with asthma outcomes than $PM_{2.5}$ mass. This hypothesis was rejected because co-pollutant models including both $PM_{2.5}$ mass and each one of the traffic-related pollutants still showed that repeated measures of FEV_1 and FE_{NO} were associated with both exposures, and these associations were largely independent of each other. We hypothesized that after adjusting for markers products of fossil fuel combustion (well represented by the EC fraction of $PM_{2.5}$), the observed independent effects of personal $PM_{2.5}$ mass may have been attributable to a variety of other PM components, including unmeasured redox active particle-bound chemicals (e.g., secondary organic aerosols), allergens, and microbial structural components or products like endotoxin that can exacerbate asthma. New data on this important unresolved issue was produced in this study. Because personal endotoxin was not positively correlated with personal $PM_{2.5}$ mass, it is unlikely that the independent effects of $PM_{2.5}$ mass on the asthma outcomes is explained by endotoxin.

The information provided in this study will support design development for additional research involving both multi-pollutant and bioaerosol monitoring in cohorts of subjects with asthma to assess the potential health impacts of combined exposures. To this end, we have submitted a proposal to NIH (R21ES019711) to study the relation of acute respiratory outcomes to personal endotoxin exposure and to address whether daily endotoxin exposure modifies the relation between repeated measurements of these outcomes with daily exposure to traffic-related air pollution or $PM_{2.5}$. The present endotoxin data will be used in the proposed study.

6. RECOMMENDATIONS

Given the results of this study, we recommend that personal endotoxin should be the exposure measurement of choice for future research on the importance of endotoxin as a risk factor for the exacerbation of asthma. Additional research is needed to assess whether home microenvironmental measurements, including vacuumed dust samples, are sufficiently representative of long-term endotoxin exposure for the assessment of chronic asthma outcomes, including the development of asthma during childhood.

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